REMARKS

Claims 1-14 are pending, claim 15 having been withdrawn in view of the Examiner's restriction requirement. Applicants nonetheless thank the Examiner for reconsidering the restriction requirement.

Applicants acknowledge the Examiner's rejection of claims 3 and 10-14, under 35 U.S.C. § 112, second paragraph, as being indefinite in view of recitation of "conditioned" (claim 3), "abnormal level of free floating DNA" (claims 10-11 and 13-14), "free floating DNA" and "total free floating DNA" (claims 11, and 12-14), and "determining the fraction of total free floating DNA" (claims 12-14). Applicants have either amended the relevant claims and/or provided appropriate rebuttal arguments to obviate these rejections.

Applicants acknowledge the Examiner's rejection of claims 1-14, under 35 U.S.C. § 112, first paragraph, as allegedly lacking sufficient enablement. Applicants have provided rebuttal arguments and claim amendments to obviate this rejection.

Applicants acknowledge the Examiner's rejection of claims 1-8, 10-11, and 14, under 35 U.S.C. § 102(b), as allegedly being anticipated by Goessl et al. (Cancer Research 2000 Vol. 60, page 5941. Applicants traverse this rejection.

Applicants acknowledge the Examiner's rejection of claims 12-13, under 35 U.S.C. § 103(e), as allegedly being unpatentable over Goessl et al., in view of Heiskanen et al. (Cancer Research 2000 Vol. 60, p. 799), as evidenced by Rein et al. (Nucleic Acids Research 1998 Vol. 26 p. 2255). Applicants respectfully traverse this rejection, based on the above described arguments in view of the Examiner's anticipation-based rejection in view of Goessl et al.

No new matter has been added.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 3 and 10-14, under 35 U.S.C. § 112, second paragraph, as being indefinite in view of recitation of "conditioned" (claim 3), "abnormal level of free floating DNA" (claims 10-11 and 13-14), "free floating DNA" and "total free floating DNA" (claims 11,

and 12-14), and "determining the fraction of total free floating DNA" (claims 12-14).

Applicants have either amended the relevant claims and/or provided appropriate rebuttal arguments to obviate these rejections.

Specifically, claim 3 has been amended to recite "treated" in place of conditioned. Support for the amendment is found in the originally filed specification, for example at page 25, bottom of second full paragraph.

Additionally, claims 10-11 and 13 have been amended to recite "increased level of free floating DNA" in place of "abnormal level of free floating DNA." Support for the amendment is found in the originally filed specification, for example at page 37, end of first carry-over paragraph, teaching in exemplary embodiments that abnormal amounts correspond to increased amounts.

Additionally, claim 12 has been amended to recite "total free floating DNA" in place of "free floating DNA" in the preamble and second step to provide for proper antecedent basis.

Applicants respectfully traverse the Examiner's rejection with respect to claim 11, because it is clear from the specification that total free floating DNA is that originating form all cell types, which is thus distinguished from "free floating DNA that originates from a specific tissue, cell type or organ."

Additionally, claim 12 has been amended to recite an additional step of "comparing the amount of DNA that exhibits a tissue, cell type or organ-characteristic DNA methylation pattern to the amount of detected total free floating DNA, thereby determining the fraction of free floating DNA that originates from the specific tissue, cell type or organ in the total free floating DNA." Support for the amendment is found in the originally filed application and claims.

No new matter has been added.

Applicants, therefore, respectfully request withdrawal of the Examiner's indefiniteness rejections.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1-14, under 35 U.S.C. § 112, first paragraph, as allegedly lacking sufficient enablement. Applicants have provided rebuttal arguments and claim amendments to obviate this rejection.

Specifically, discussing the *WANDS* factors, and reciting the various elements of Applicants' claims, the Examiner states that the specification and art do not enable determination of the presence or absence of ANY diseased condition in a tissue, cell type of organ, or to detect the presence or absence of a diseased condition by determining the amount or presence of free floating DNA without any methylation step, particularly in an unpredictable art.

The Examiner states that the specification teaches that characteristic methylation patterns can be positively correlated with specific organs, tissues and cell types, for humans, but not for all animals nor all diseases. The Examiner urges that methylation patterns are not reproducible (citing Ziegler), that genetic variations and associations are often irreproducible (citing Hirschhorn), unpredictability inassociating circulating DNA with disease (citing Bremnes, Jung, and Sidransk). The Examiner concludes that despite the level of skill in the art being high, given the specification guidance and working example, it would require undue experimentation to practice the invention as claimed.

Applicants respectfully traverse the Examiner's enablement rejection in view of Applicants' present claim amendments, and in view of the applicable law.

Applicable Law

Alleged lack of enablement. The test for sufficient enablement is whether the amount of experimentation required by one of skill in the art to make and use the claimed subject matter is not *undue* (although a reasonable amount of experimentation is permitted). The test for undue experimentation requires an application of the factors set forth in *In re Wands*, cited by the Examiner.

Analysis

First, the examiner urges that neither the art nor the specification enables one to determine the presence or absence of ANY diseased condition in a tissue, cell type or organ.

Applicants have amended the claims to recite detection of the *presence* of a "*cell proliferative disease*."

Second, the Examiner urges that the specification fails to enable one of skill in the art to detect the presence or absence of a diseased condition by determining the amount of or presence of free floating DNA without any methylation step. Applicants disagree, because the art teaches that at least an increased level of free floating DNA in blood has been correlated with existence of cellular proliferative disease (cancer) in humans.

Applicants have, nonetheless, amended the claims to recite a step of performing a methylation analysis; that is "analysing for a DNA methylation pattern that is characteristic for the particular tissue, cell type or organ."

At page 5 the Examiner states that the methods claimed (1-14) merely detect free floating DNA, wherein the specification is drawn to methylation steps. Applicants respectfully direct the Examiner's attention to the fact that the method is not simply to be understood as the "determining the amount of or presence of free floating DNA" but the "determining the amount of or presence of free floating DNA, *which originates from a specific tissue, cell type or organ*," which is a subpart of the total amount of free floating DNA, that may be determined by any means. It is the recognition of *tissue, cell type or organ-characteristic* DNA that is a key step of the claimed inventive methods. This step may be performed by recognition of *tissue, cell type or organ-characteristic* methylation patterns, which are independent of disease (rather than being specific for cancer). These specific methylation patterns enable the person skilled in the art to identify an increased level of DNA, derived from a specific organ (tissue, cell type), in the blood. Only from this it is deduced that a cell proliferative disease (related to this organ (tissue, cell type)) may be present.

At page 6 the examiner states that the specification does not provide any correlative

associations with broad detection of ANY disease. Applicants respectfully point out that the invention is not centered on direct correlation of disease type with methylation patterns. Rather, a correlation of organ (tissue or cell-type) with methylation patterns of DNA isolated from such organs (tissue or cell-type) is described, by reference to the art. This has been published elsewhere (for example, by the Human Epigenome Project (http: // www.sanger.ac.uk / PostGenomics / epigenome /) Eckhardt et al. in Nat Genet. 2006, or Rakyan et al. PLoS Biol. 2004;2; e405). In addition, increased levels of DNA have been correlated with the presence of cell-proliferative disease (*i.e.* a disease shedding DNA into the blood stream or other body fluids). Significantly, however, it is only by recognizing the potential of the organ specific methylation patterns for the identification of the source of said free floating DNA, that the inventive method provides for a correlation of increased levels of specific-organ derived DNA with presence of cell-proliferative disease (*i.e.* a disease shedding DNA into body fluids) of said specific organ. For instance, the following example serves to illustrate aspects of the inventive methods:

a) *body fluid sample analysis (by any suitable means to detect DNA levels) reveals an increased amount of total free floating DNA*
b) *deduction is made that the corresponding patient is suffering from a cell proliferative disease (as has been indicated in the art)*
c) *methylation analysis of (isolated) total free floating DNA reveals lung specific methylation patterns, based on comparison of methylation pattern detected with methylation patterns known to be characteristic for several organs*
d) *quantitative analysis reveals level of lung derived DNA in total amount of free floating DNA is increased*
e) *deduction is made that the lung of said patient is suffering from a disease, spreading DNA into the body fluid (i.e. cell proliferative disease of the lung).*

At page 7, the Examiner urges that "it is unpredictable that ANY disease would be detectable in free floating DNA because it is unclear if any tumor, organ, or tissue can be detected

in a fluid sample.

Applicants, as stated above, have amended the claims to recite *cell proliferative disorders*, for which it can be reasonably concluded, that an organ or tissue affected by such an disorder will shed DNA into a body fluid where it can be detected. Furthermore, at page 7, the Examiner inappropriately interprets Applicants' sentence "to validate this a next step *could be*" as "a validation *must* be performed". Applicants object, because Applicants merely suggested that a further validation *could be* useful.

At page 8, the eExaminer urges that the specification indicates that [..] a comparison study *must* be done. Applicants respectfully disagree, because it is not *necessary*, to detect the presence of a disease, because tissue specific methylation patterns are a recognized part of the art, and being publicly available one or ordinary skill can correlate the obtained methylation pattern to the published ones. The mere detection of, for example, an increased amount of lung specific methylation pattern in DNA obtained from a body fluid of a human individual, would be sufficient to conclude that the individual suffers from a lung cell proliferative disorder. It is obvious that the diagnosis will be improved the more data are accessible relating to the field, however the general principle has been described taught and enabled in the present specification, and in view thereof, it is obvious to the person skilled in the art how to perform such a method for detecting the presence of a cell proliferative disorder.

Moreover, the claims have been amended to recite a *comparing* step.

At page 9, the Examiner urges that "the art teaches that using circulating DNA as a diagnostic tool is unpredictable and that methylation patterns are not reproducible." Applicants point out that the Examiner has failed to provide adequately representative citations or a reasoning upon which to base the Examiner's conclusion. In fact, there are a number of clinically valid studies published, which demonstrate the reproducibility of methylation patterns. For example, to cite but the most recent development in the field, the Applicants point out that Abbott, a leading player in the field of molecular diagnostics, spent a reasonable amount of money to develop a molecular diagnostic methylation marker for early diagnosis of colon cancer (see press release).

The company Epigenomics alone, for example, has demonstrated in multiple clinical case control studies with about 3,000 blood plasma samples from colorectal cancer patients, healthy controls, and patients with non-cancerous colon diseases that methylated DNA of Septin 9 shed by tumors into the blood stream can serve as a biomarker for the sensitive and specific detection of colorectal cancer. Another example is a recent publication presented at ECCO - the European Cancer Conference, Barcelona, Spain. (ECCO14 Abstract 4.009), also press released at the 18th of September 2007; where the poster presented reports on a study wherein a series of matched urine and plasma samples was analysed. Herein methylation markers that discriminate prostate cancer patients from healthy controls were identified. Also, these data have been further confirmed in a second clinical study of 38 prostate cancer and 38 biopsy negative patients and 64 age-matched healthy control individuals. Therefore, there is no support for a conclusion that circulating DNA, as a diagnostic tool, is unpredictable and that methylation patterns are not reproducible.

With respect to the correlation between tissue specificity and methylation (which is a key element of the inventive methods), the Applicants refer (in the specification) to Adorjan et al. (2002, Nucleic Acids Res. 30, e21), who described the correlation of specific methylation patterns for healthy tissue for the first time. But especially the results of the Human Epigenome Project (HEP), which one of ordinary skill in the art is certainly familiar with, give evidence for the fact that tissue specific methylation exists, independently of disease status. Source (27.09.2007) :

http://www.sanger.ac.uk/PostGenomics/epigenome/

> "One of the aims of the Human Epigenome Project (HEP) is to generate *tissue-specific DNA methylation reference profiles* of the human genome. The chosen approach involves treatment of the genomic DNA with sodium bisulphite which converts unmethylated cytosines into uracil but does not affect methylated cytosines. Following PCR amplification and sequencing of selected amplicons from bisulphite-converted DNA, the degree of methylation can be determined by comparison of the corresponding signal ratios at CpG dinucleotides, the predominant sites of DNA methylation.
>
> Release 7th Oct 2003 comprised about 135,000 CpG methylation values, obtained from the analysis of 235 amplicons across the 4Mb major histocompatibility complex on chromosome 6 in 32 samples (*derived from 7 different tissues*). The data and results of this pilot study are described in:-

DNA methylation profiling of the human major histocompatibility complex: a pilot study for the human epigenome project.
Rakyan VK, Hildmann T, Novik KL, Lewin J, Tost J, Cox AV, Andrews TD, Howe KL, Otto T, Olek A, Fischer J, Gut IG, Berlin K, Beck S
PLoS Biol. 2004;2;e405. PMID: 15550986

In response to the quotations made from Ziegler et al. (page 9 of office action), Applicants would like to point out that the problems reviewed relate to cancer markers (as opposed to tissue (or organ markers) and are therefore not applicable as such. For example, it seems apparent, that the number of lung cancer cells is much smaller than the number of affected lung cells, which might shed their DNA into the blood. Hence detection of *lung cancer* DNA might be more difficult than detection of *lung tissue* DNA. Also the methylation in a cancer cell might not be co-methylated to the extent assumed so far, and hence the detection assay employed might fail to detect the cancer DNA. This would be expected to be different for tissue specific methylation patterns, which seem to be co-methylated in extended regions.

Second, the fact that some "other genes were shown to be not significantly associated with the presence of cancer" and that contradictory results may have been in the art (for other cancers as well) is not surprising, as a number of insufficient methods have been used, that resulted either in false positives when the method wasn't specific enough or the results represented false negatives when the method was not sensitive enough.

In response to the "In summary, ..."-paragraph starting at the bottom of page 9, Applicants would like to emphasize that the amended claims no longer encompass the detection of *any* disease using samples from *any* individual by the detection of free floating DNA.

In response to the assertions by the Examiner relating to the unpredictability of the art, Applicants would like to point out that certainly statements as cited by the examiner made by Hirschhorn et al. raising a cautionary alarm when considering the use of results from a single report on a genetic variance being correlated with disease as diagnostics; or Bremnes et al. that "large perspective clinical studies are needed to validate and standardize any test for DNA alteration in plasma or serum [...]" are relevant, when discussing the approval of a diagnostic test

to the market by a regulation office such as the FDA, however it can not be a requirement of enablement of a patent, to present the results of such a large study, which is a tremendous effort and time and cost intensive.

For what concerns Jung et al., Applicants thank the examiner for drawing attention to this paper, as his results underline the importance of the inventive method. The problem in the state of the prior art may be regarded as the limited information that is achievable from detecting the amount of *total* free-floating DNA in plasma, as evidenced by Jung. T he inventive method overcomes the problem in the state of the art by providing for a method to determine the origin of said free floating DNA and enable the practitioner to draw informative conclusions from that. The inventive concept comprises a general concept, and a large improvement to the state of the art.

The Examiner urges that circulating DNA is not always correlated with the presence of cancer in a subject. The claims have been amended to not comprise the detection of absence of disease anymore. Significantly, there is no report known showing that elevated levels of circulating DNA, predominantly derived from a specific organ, was detected in perfectly healthy individuals. Furthermore, in response to the quotation of Sidransky (page 11 and 12), "the origin of circulating DNA in the blood is uncertain," the Applicants respond that the problems he describes are exactly those that the inventive method overcomes.

As stated by the Examiner :

"The current art teaches [...] that methylation can be detected in normal tissue. This indicates that detection of methylation does not indicate neoplastic tissue." Exactly. That is why the inventive method determines not only whether tissue specifically methylated DNA is present, but whether it occurs in a body fluid (where it normally should be absent), thereby indicating a diseased state of the tissue or organ, which apparently sheds its DNA into said body fluid. The fact that aging effects methylation patterns as well, should been taken into consideration when designing the experiments to identify tissue specific markers. This is part of the HEP project and not relevant for the inventive method, which makes use of tissue specific markers, and is not about identifying them.

In summary, Applicants refuse all of the assertions made by the examiner (at page 12, 13):

The method is no longer claiming the detection of *any* disease.

The specification does not disclose the ***need*** of a validation study, but simply suggests this *could be* done.

Detection of disease with methylation patterns in free floating DNA is *not* unpredictable, as can be seen at least from the validated colon cancer marker Septin 9, for methylation has repeatedly and predictably been correlated to cancer, and which is currently developed to become an approved blood based colon cancer marker.

Based on these unsupportable assertions, the conclusion cannot be drawn that the skilled artisan would have to perform undue experimentation to determine the correlation of disease detection *of a specific organ or tissue* (!) to detection of *organ or tissue specific* free floating DNA, which is a key element of the invention, and not simply the correlation of any disease with any free floating DNA. Hence the correlation has to be made, for example, that lung specific methylation patterns detected on circulating DNA in, for example blood samples, correlate to a diseased lung; and colon specific methylation patterns detected on circulating DNA in, for example urine samples, correlate to a diseased colon. The skilled artisan will be able to draw this conclusion without undue experimentation.

Applicants, therefore, respectfully request withdrawal of the Examiner's enablement rejection of the amended claims.

Rejections under 35 U.S.C. § 102

The Examiner rejected claims 1-8, 10-11, and 14, under 35 U.S.C. § 102(b), as allegedly being anticipated by Goessl et al. (Cancer Research 2000 Vol. 60, page 5941.

Specifically, the Examiner urges that Goessl teaches determining prostate cancer based on the presence of GSTP1 in bodily fluids.

Applicants traverse this rejection.

Applicants respectfully point out that the Examiner may have misconstrued the present

invention, based on the use of organ markers or tissue markers, which are nucleic acids bearing organ or tissue specific methylation patterns, independent from the question whether the organ or tissue is diseased or not. Whereas, the prior art merely teaches the use of cancer markers, such as for example GSTP1, which is a nucleic acid bearing a cancer specific methylation pattern.

The teaching of Goessl et al. differs from the claimed method, at least in the fact that GSTP1 is a nucleic acid which bears a methylation pattern that is *specific for a type of cancer*, such as prostatic carcinomas. It is not used as a marker for the prostate organ. The difference being that GSTP1 is a) also known to be methylated in other similar cancer types of different organs, for example breast and renal carcinomas, as reported by Esteller et al. in Cancer Research 58, 4515-4518 (Abstract), and b) the methylation pattern of GSTP1 detected by Goessl et al. was not even detectable in prostate tissue from individuals not suffering from prostate cancer. Hence without a medical history indicating a diseased prostate, detecting GSTP1 methylation (as described by Goessl) would only provide the information that the patient might suffer from a type of cancer, such as prostate cancer.

Therefore, it is incorrect to say "Goessl et al. teaches the detection of a methylation pattern to determine the presence of DNA from prostate tissue," because this could only be provided by identifying a prostate (organ) specific methylation pattern on the GSTP1 gene, which is not methylated in the same way in other organs (diseased or not).

Detecting a methylation pattern in the circulating DNA which is *characteristic for a specific organ or tissue, such as prostate* however, is for the first time taught and disclosed in the current invention. The differentiating feature of the methods as claimed in all the independent claims therefore is in claims 1 and 2:

- determining an amount or presence of free floating DNA *that originates from a particular tissue, cell type or organ* in the sample comprising analysing for a DNA methylation pattern that is characteristic for the particular tissue, cell type or organ;

in claim 8 (old 11)

- determining an amount or presence of free floating DNA *that exhibits a DNA methylation pattern characteristic of a particular tissue, cell type or organ*

in claim 9 (old 12)

- determining an amount of free floating DNA that *originates from a specific tissue, cell type or organ* by determining an amount of free floating DNA that exhibits a DNA methylation pattern characteristic of a tissue, cell type, or organ

and in claim 10 (old 13)

- analysing several methylation-specific positions in the treated DNA, and thereby *determining an amount of DNA that exhibits a tissue, cell type or organ-characteristic DNA methylation pattern*;

This difference is essential and has neither been taught nor anticipated by Goessl et al. , nor by Goessl as evidenced by Rein et al..

Applicants, therefore, respectfully request withdrawal of the Examiner's anticipation rejection, based on Applicants' claim amendments and rebuttal argument described herein.

Rejections under 35 U.S.C. § 102

The Examiner rejected claims 12-13, under 35 U.S.C. § 103(e), as allegedly being unpatentable over Goessl et al., in view of Heiskanen et al. (Cancer Research 2000 Vol. 60, p. 799), as evidenced by Rein et al. (Nucleic Acids Research 1998 Vol. 26 p. 2255).

Applicants respectfully traverse this rejection, based on the above described arguments in view of the Examiner's anticipation-based rejection in view of Goessl et al. On the basis of Goessl et al. in view of Heiskanen et al. as evidenced by Rein, alone or in combination, there is absolutely no suggestion whatsoever to use a tissue or organ specific methylation marker. These references do not teach determining an amount of free floating DNA that originates from a specific tissue, cell type or organ by determining an amount of free floating DNA that exhibits a DNA methylation pattern characteristic of a tissue, cell type, or organ. Nor do they teach determining an amount of DNA that exhibits a tissue, cell type or organ-characteristic DNA

methylation pattern.

Applicants, therefore, respectfully request withdrawal of the Examiner's obviousness rejection, based on Applicants' claim amendments and rebuttal argument described herein.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request entry of the present Amendment and allowance of the amended claim set provided herein. The Examiner is encouraged to phone Applicants' attorney, Barry L. Davison, to resolve any outstanding issues and expedite allowance of this application.

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